applicant's disclosure as a recipe from which to choose references that describe each of the ingredients:

> Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references. *See, e.g. C.R. Bard, Inc. v. M3 Sys., Inc.,* 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed. Cir. 1998)(describing "teaching or suggestion or motivation [to combine]" as an "essential evidentiary component of an obviousness holding").

In re Dembiczak, 50 USPQ2d 1614 (Fed. Cir. 1999). The court states that "actual evidence" of a motivation to combine references is required, "[t]hat is, the showing must be clear and particular. *See, e.g., C.R. Bard,* 157 F.3d at 1352, 48 USPQ2d at 1232. Broad conclusory statements regarding the teaching of multiple references, standing alone, are not 'evidence.'" *Id.*

Applicants respectfully submit that, in setting forth the instant rejection, the Examiner has not satisfied the requirement of providing actual evidence, from the prior art, providing motivation for making the claimed invention and of a reasonable expectation of success for practicing the claimed invention.

Masuta et al.

The Examiner argues that Masuta et al teaches "the use of a vector comprising satellite RNA of a plant virus, ...particularly wherein the satellite RNA may be derived from a satellite virus, including both STNV and STMV, or wherein the satellite RNA may be contained within helper virus particles...". Applicants respectfully disagree. The terms 'satellites of plant viruses', 'satellite RNA of a plant virus, and 'satellite (RNA) virus' have distinct meanings that are clear to one of ordinary skill in the art. The Examiner's attention is respectfully directed to the attached Declaration by Dr. Frank Meulewaeter under 37 CFR

§ 1.132, which confirms that these expressions have distinct meanings. Further, Applicants also submit the following publications:

a) A. Simon, *Plant Molecular Biology Reporter,* Vol. 6(4), 1988, p240-252

P240, lines 1-11:

> "*Satellite RNAs are small, single-stranded RNAs* which require a helper virus for their replication, and *are encapsidated in their helper viral coat protein* [*i.e.* together with the helper virus genomic RNA].... *Unlike satellite viruses which encode for their own coat protein* [*i.e.* are not encapsidated together with the helper virus genome], most satellite RNAs contain no open reading frames of appreciable length...."

[emphasis added]

b) Qiu and Scholthof, *J. Virology,* June 2001, p5429-5432

P4529, paragraph 1:

> "To date, three types of subviral agents have been described that require a helper virus to complete their life cycles: satellite viruses, satellite RNAs (satRNAs), and defective-interfering RNAs (DIs). Satellite viruses encode a capsid protein (CP) which is used for encapsidation of the RNA genome to form icosahedral particles. .... *satRNAs represent another species of small RNA molecules* ... .

[emphasis added]

Consistent with the Declaration of Dr. Meulewaeter and the publications cited above, the Masuta et al publication distinguishes two categories of 'satellites' (col 1, lines 16 -17):

1. Satellite viruses, such as STMV and STNV (col1 lines 18-22) and
2. Satellite RNAs of plant viruses, such as satellite RNAs of CMV (col1, lines 23-27).

Masuta et al suggest only to use satellites of the second category, *i.e.* satellite RNAs of plant viruses, as vectors for expressing proteins or expressing antisense sequences of

viruses. In contrast, the present claims are drawn to vectors derived from satellite RNA viruses, and not to vectors derived from satellite RNAs of plant viruses. There is thus no motivation provided by the cited art to modify the disclosure of the Masuta et al publication, nor to combine it with the disclosure of Grierson et al., to arrive at the presently claimed invention. Nor does Masuta et al. provide one of ordinary skill in the art with the requisite reasonable expectation of success for one of ordinary skill in the art to make such modifications and/or combinations in order to arrive at the presently claimed methods and kits.

Grierson et al.

The Examiner argues that the constructs disclosed by Grierson et al comprise both an antisense and a sense portion, wherein upon transcription said constructs yield inhibitory RNA, which comprises both a sense and an antisense region. However, Grierson et al neither discloses nor suggests the use of satellite RNA viruses as vectors to introduce constructs yielding inhibitory RNA into a plant cell in order to silence endogenous plant genes. The Grierson et al. publication likewise fails to provide either motivation or a reasonable expectation of success for one of ordinary skill in the art to use satellite RNA viruses as vectors for silencing of endogenous plant genes.

Moreover, the present claims are not limited to a particular silencing technology, such as antisense suppression, sense suppression (co-suppression), or both sense and antisense (inverted repeats). Indeed, the present claims provide one of ordinary skill with the choice of using non-autonomous satellite RNA viruses (satellite RNA viruses), rather than autonomously replicating and spreading RNA viruses, as vectors for delivering inhibitory RNA into the cytoplasm of plant cells. The choice of modifying satellite RNA

viruses, rather than autonomous RNA viruses (such as the helper virus genomes), into gene-silencing vectors is not an obvious choice for the reasons discussed herein.

Fitzmaurice et al.

Applicant submits that the presently claimed invention is not *prima facie* obvious over either Fitzmaurice et al alone, or in combination with Grierson et al. There is no motivation in the prior art for one of ordinary skill to modify and/or combine the teachings of these publications in order to arrive at the presently claimed invention. Nor do the cited publications provide one of ordinary skill in the art with the requisite reasonable expectation of success. Neither Fitzmaurice et al nor Grierson et al either disclose or suggest whether or how the vectors of Fitzmaurice et al can be modified to silence endogenous plant genes, as in the present claims.

1. No motivation to modify/combine

Fitzmaurice et al. discloses over expressing proteins in plant cells, and fails to over express a heterologous protein in a plant cell using STMV based vectors. From this disclosure, there is no reason why one of ordinary skill in the art would be led to conclude that satellite RNA viruses can be or should be modified to silence endogenous plant genes.

There are only two places in the Fitzmaurice et al publication where its authors refers to possible uses of STMV based vectors for purposes other than overexpression of genes. The first is on page 10, lines 13-19, where Fitzmaurice et al. propose to introduce an antisense RNA of a plant virus into a plant cell in order to make the plant resistant against virus infection. The viral vector would thus target a nucleic acid located in the cytoplasm of

the cell (namely the viral genome), and not in the nucleus, as in the present claims. The second part is found on p13, lines 30-37:

> An exogenous RNA segment *may* also be an anti-sense RNA to a nucleic acid segment that *occurs naturally in a target cell,* or that *may* occur in a target cell if the cell is infected with a virus, and whose *function* is intended to be blocked, interrupted or otherwise interfered with *by hybridization* with the recombinant STMV genome of the invention which comprises the exogenous, anti-sense segment.

[emphasis added]. Applicant submits that it is unclear what is meant by this sentence. Specifically, it is unclear what a nucleic acid segment is that "occurs naturally in a target cell" (a DNA segment? an RNA segment? in which compartment of the cell would it be located?), nor is it clear what is meant by its "function" (transcription? translation? phenotypic change?) or "by hybridization with the recombinant STMV genome" (RNA-RNA hybrid? RNA-DNA hybrid?).

Applicants respectfully submit that the Fitzmaurice et al. publication has to be interpreted in view of the state of the art at the time of its publication, 18 October 1990. It was not known in the art until the publication of Kumagai et al (1995, *PNAS* Vol.92, pp1679-1683 / WO9534668) that cytoplasmic antisense RNA can downregulate endogenous gene expression of nuclear encoded genes. Consequently, this sentence from Fitzmaurice et al. must be interpreted as referring to the creation of plants resistant to virus infection (by introducing antisense RNA of viral genes either into the cytoplasm (for cytoplasmic viruses) or into the plant genome for nuclear viruses), and not to the silencing of nuclear encoded plant genes, as presently claimed. Further, Applicants would like to note, that in the vectors of the instant claims, the mRNA of the target gene cannot hybridize with the sense RNA of the recombinant STMV genome, as is suggested for the vectors of Fitzmaurice et al.

Additionally, Applicants respectfully direct the Examiner's attention to the later publication of Fitzmaurice et al. (Mirkov et al, *Virology* 1990, 179, 395-402; attached), which contains no suggestions of possible alternative uses of their vectors (other than for overexpression). In fact, this later publication highlights potential problems associated with STMV-based vectors, such as the accumulation of mutations and recombination between the vector and the helper virus genome. This recitation of potential problems cannot be seen as providing either motivation to use such vectors as tools for silencing of nuclear plant genes, or a reasonable expectation of success in employing the vectors in such a manner. Consequently, one of ordinary skill in the art would conclude that the vectors of Fitzmaurice et al. are unworkable for gene silencing (this can equally be concluded from the publication of Routh et al. 1995, *Virology* 212, pp121-127, cited below, attached).

2. Lack of a reasonable expectation of success

Fitzmaurice et al. states on page 36, lines 17-22 that only one out of four plants were infected with the vectors comprising CAT nucleic acid and that the recombinant STMV vector "is weakly infective in combination with helper U5." The CAT nucleic acid was inserted into the 3' end of the coat protein coding gene. Applicants submit that, from this paragraph, one of ordinary skill in the art would doubt the functionality of the vectors of Fitzmaurice et al. In a later publication by Fitzmaurice and others (Routh et al. 1995, *Virology* 212, 121-127; attached) it is shown, in confirmation with Fitzmaurice et al., that a) the number of plants in which deletion and frame shift STMV mutants were able to replicate was lower than for wild type STMV and much less RNA accumulated (p. 124, col. 1, ¶ 2);

b) in growth chambers mutant STMV RNA could generally only be detected in the inoculated leaves (p. 124, col. 1, ¶ 2); and

c) co-inoculations of STMV coat protein mutants and helper virus resulted in severe modification of the phenotype: severe veinal chlorosis, necrotic flecking of systemically infected leaves (p 124, col 2, lines 1-14).

The Routh et al. 1995 publication also states (p 121, col 2, line 10-18), that

> "..there are no data that define the sequences essential for accumulation and movement of the STMV ... . The present study was undertaken to determine which regions of the STMV genome are required for efficient accumulation and systemic movement and if RNA sequences in the interior of the genome could be removed for possible replacement with foreign genes in order to use STMV as a broad plant host range recombinant expression vector."

This statement confirms that the Fitzmaurice et al. publication cited by the Examiner does not teach how to construct efficient expression vectors using STMV, as otherwise there would have been no need for the Routh et al. study published in 1995 by Fitzmaurice and others.

Applicants submit that one of ordinary skill in the art would not have had a reasonable expectation of success and would not have been motivated to modify the vectors of Fitzmaurice et al. considering the indications in the literature and in Fitzmaurice et al. that modifications may lead to weak infectivity, much less vector RNA (maybe even no vector RNA in upper leaves) and necrosis phenotypes, all of which would make it very unlikely that a gene silencing phenotype would be induced or, if induced, would not be masked by a chlorosis/necorosis phenotype.

Further evidence

Despite the Examiner's opinion that the use of satellite RNA viruses as gene silencing vectors is an obvious choice in hindsight, Applicants submit that the use of satellite RNA viruses was *not an obvious choice* at the time of the instant invention. At the time of filing of the invention it was generally believed in the art that satellite RNA viruses would not be a good choice for constructing vectors, as certain properties associated with satellite RNA viruses, such as lack of stability, high mutation rate and a cytoplasmic mode of replication, which depends on helper viruses (i.e. is *non-autonomous*), were believed to make satellite RNA viruses unsuitable as vectors for silencing of plant genes. Persons skilled in the art chose instead to modify *autonomously* replicating RNA viruses, such as for example TMV and ToMV (Kumagai et al 1995, *PNAS* 92:1697-1683) or PVX (Ruiz et al 1998, *The Plant Cell* 10: 937-946), into gene silencing vectors, which did not have the alleged negative properties associated with satellite RNA viruses.

In view of the evidence submitted herewith by Applicants, it is clear that persons skilled in the art were *not motivated* to use satellite RNA viruses as tools for silencing endogenous plant genes, but rather used autonomous RNA viruses, and that there was *no reasonable expectation of success* in using satellite viruses as vectors for silencing of endogenous plant genes:

1. The scientific literature to date lists viruses which have been used and which are suitable, or are suggested as being suitable, as gene silencing vectors for plants. *See, e.g.,* Linbo, Fitzmaurice and della-Cioppa (*Current Opinion in Plant Biology* 2001, 4:181-185); Baulcombe (*Current Opinion in Plant Biology* 1999, 2:109-113). Satellite RNA viruses have not been suggested in the prior art as suitable tools for silencing nuclear encoded plant genes.

2. Applicants submit that the literature has provided disincentives to use satellite RNA viruses for silencing of endogenous plant genes.

First, many more autonomously replicating plant RNA viruses have been well characterized than satellite RNA viruses, of which only 4 have been characterized to date (STMV, STNV, SMWLMV and SPMV, see Qiu et al, *J. Virol.* 2001, Vol.75, p5429, col 1, lines 6-10, attached). Besides a much larger choice of autonomous RNA viruses, it was exactly their ability to replicate as autonomous entities inside the plant, which was seen as an *advantage* when considering viruses suitable for manipulation. See, for example, Donson et al 1991 (*PNAS* 88:7204-7208) and by Joshi & Joshi 1991 (*FEBS Lett.* 1991, 281(1,2):1-8).

Second, in addition to the publications cited above, the following publications teach away from choosing satellite RNA viruses (such as STMV) as gene silencing vectors. Also, certain characteristics of autonomous helper viruses, such as TMV, are reported in these publications as advantageous, providing a motivation to use helper viruses as vectors:

(a) Fernando Garcia-Arenal (*Ann Rev Phytopathol.,* 2001; attached), p. 173, lines 33-35:

"Nevertheless, laboratory stocks of STMV are *twice as variable* as those of TMGMV (98,156), suggesting higher constraints to variation for the helper virus than for the satellite virus." [emphasis added].

(b) Kurath and Dodds (*RNA,* 1995, 1:491-500; attached) showed that, when STMV is passaged through plants, single base mutations, substitutions and deletions accumulate, confirming that STMV replication generates heterogeneity and diversity quickly and with high frequency. Replication error "hot spots" or "clusters" were reported in the STMV genome, while no such hot spots have been found in the helper virus genome TMV.

(c) Kurath, Rey and Dodds (*J. Gen. Virol.,* 1993, 74:1233-1243; attached) showed that the helper virus acts as a selection pressure on STMV and that STMV is capable of very rapid genetic modification in response to selection pressure.

(d) Kurath, Heik and Dodds (*Virology* 1993 194:414-418; attached), p417: "High level of genetic diversity of STMV from small geographic area is in contrast with the low diversity found in populations of its helper virus."

(e) Kearney et al (*Virology* 192:11-17, 1993, attached) show that, in contrast to STMV, the helper virus TMV accumulates mutations at a very low rate and that there are no indications of replication error "hot spots" present in TMV. Such "hot spots" are reported to be present in STMV.

(f) Rodriquez-Cerezo and Garcia-Arenal (*Virology* 170:418-423, 1989; attached) report that TMV is less heterogeneous and has a higher genetic stability than other RNA viruses.

Conclusion

Applicants respectfully submit that a *prima facie* case of obviousness has not been made out, as the Examiner has not shown a motivation in the prior art to modify and/or combine the disclosures of the cited publications to arrive at the presently claimed inventions, and because none of the cited publications provides the requisite reasonable expectation of success in making and using the presently claimed invention. Applicants therefore respectfully request withdrawal of the rejections.

From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order, and such action is earnestly solicited.

In the event that there are any questions concerning this paper, or the Application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of the Application may be expedited.

Respectfully submitted,

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